Varietal Differences in Amylose Content of Rice Starch

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The study of the amylose content of a number of southern rice varieties was undertaken as part of a more extensive investigation of quality control in rice products. These analyses have revealed a consistent variation of amylose content with grain type, the longer grain varieties having the higher amylose content. Possibly, this difference in starch composition is responsible for the known differences in cooking quality. Studies on samples of different seeding dates have so far revealed no significant differences in amylose content attributable to date of planting.

Of the various important sources of dietary starch, rice starch has been subjected to the least extensive study in the United States. The most precise physicochemical investigations on starch have been conducted in England by Brown and coworkers (*3*) and Hirst and Young (*5*). In Japan and India, chemical studies on different aspects of rice starch composition and characteristics have been conducted for the last 20 years or so. Worthy of special mention is an extensive study of the amylose and amylopectin content of Indian rices conducted in 1951 by Rao, Murthy, and Subrahmanya (*9*).

In the United States, the variety of traditional practices employed in India in the treatment of rice prior to cooking are not observed, but among the domestic varieties there are distinct differences in cooking quality which, in some instances, have affected their acceptance for specific purposes. According to Jodon and de la Houssaye (*6*), the quality of cooked rice varies from "very sticky" to "flaky," the short- and medium-grain varieties being somewhat sticky, whereas the long grains are usually flaky. Cooking quality is also described as "moist" or "dry." There is considerable demand for the slightly sticky or moist type in the South, but in other sections the flaky or dry type probably is preferred by consumers. Any correlations which might be obtained between chemical composition and processing behavior would be of obvious value to the food processing industry, as well as to the rice breeding program. For these reasons, an investigation of varietal differences in amylose content was undertaken.

Experimental

Amylose Determination. The principal difficulty encountered in the chemical analyses was to establish a method for amylose analysis which would give reproducible results on control samples. The following outline describes the procedure which was finally adopted.

The rough rice was dehulled and then milled in a standard milling machine. The milled rice was ground to 60-mesh and defatted with absolute methanol. The methanol was mixed with the ground rice and the suspension allowed to stand for 2 to 3 hours. This solvent was then decanted, fresh alcohol was added, and the suspension was allowed to stand overnight. At the end of this period, the solvent was decanted, the remainder of the alcohol was removed with suction, and the sample was dried in a vacuum desiccator. This procedure avoided any lipide interference in the subsequent iodine color development.

The dried rice was then ground to 80-mesh and its moisture content was determined.

For amylose determination, an adaptation of the method of McCready and Hassid (*7*) was used. The finely ground rice was difficult to solubilize, and a long soaking treatment was necessary to obtain satisfactory agreement between duplicate samples and with daily control samples. A 100-mg. sample of the ground rice was placed in a 100-ml. volumetric flask with 1 ml. of 95% ethyl alcohol and 10 ml. of 1N sodium hydroxide. The sample was refrigerated at 4° C. for 22 to 24 hours, after which time water at the same temperature was added to the 100-ml. calibration mark, and the flask contents were well mixed. The sample was then allowed to stand for 16 to 18 additional hours at 4° C. When a number of samples were analyzed daily, it was a simple matter to organize the routine so that no inconvenience was experienced by the long soaking times.

For the development of the blue starch-iodine color, a 5-ml. aliquot of the alkaline solution was pipetted into a beaker, 50 ml. of water were added, and 0.05N hydrochloric acid was added with stirring to a predetermined arbitrary pH meter reading (in this case, a reading of 10.5 proved most satisfactory). A true pH reading was not obtained in the presence of the colloidal starch solution, but it was necessary with these samples to adjust the pH carefully to some arbitrary standard so that the pH-sensitive, starch-iodine blue color would be reproducible. An arbitrary pH reading of 10.5 corresponded to an actual pH near neutrality, as well as could be estimated with indicators. Apparently, this pH sensitivity is not encountered with pure starch samples, but was a constant problem with ground rice samples. Following the pH adjustment, 2 ml. of iodine reagent was added (0.2 gram of iodine and 2.0 grams of potassium iodide diluted to 100 ml. with distilled water), the solution was made up to volume in a 100-ml. volumetric flask, and the absorbance was read at 590 mμ on a Klett-Summerson colorimeter. The absorption spectra of the amylose–iodine blue color and the amylopectin–iodine red color were checked on the Beckman Model DU spectrophotometer, and a wave length of 590 was selected as most suitable for the amylose determination. For each rice sample, weighed duplicates were taken, and duplicate aliquots were removed from each flask following the alkaline solubilization. Control samples were run daily to check on reproducibility.

A standard curve was determined with pure rice amylose prepared by the method of Schoch (*8*). Pure amylopectin was not prepared, but the absorption spectrum of the amylopectin–iodine red color was determined from a sample of waxy rice.

Total Starch Determinations. Total starch was determined by a reducing sugar analysis of ground, defatted samples which had been subjected to enzymatic and acid hydrolysis, according to the official AOAC method (*1*). The glucose values were converted to starch values by applying the proper correction factor.

Calculations. The percentage amylose values were obtained by dividing the amylose content of the 100-mg.

samples by the corresponding total starch content, both corrected for dry weight.

Results and Discussion

Varietal Differences among Louisiana and Texas Samples. Table I gives the percentage amylose for samples of typical Louisiana varieties obtained from the Rice Experiment Station at Crowley, La., and a second series of samples obtained from the Rice-Pasture Experiment Station, Beaumont, Tex. The latter series of samples is of particular interest, as it has been analyzed in three independent laboratories—each laboratory using a different method of study. These three sets of data are given in Table I. The original comparisons were made by Halick, using his newly developed rapid starch–iodine blue test (*4*). Halick's method is not proposed by him as an amylose analysis but gives a comparative value which may be correlated with amylose values obtained by other methods. When Halick's percentage transmittance values are converted to absorbances, a coefficient of correlation of 0.916 is obtained for the LSU amylose data and Halick's data (the Century Patna 231 was omitted in the calculations because it gives an abnormally high iodine blue value).

Amylose determinations were made in the author's laboratory by the method described earlier and by Hogan at the Southern Utilization Research and Development Division, U. S. Department of Agriculture, New Orleans, La., using the potentiometric iodine method of Bates, French, and Rundle (*2*). The agreement obtained by the two methods is striking (Table I).

The general trend exhibited by the samples shown in Table I is that of a higher amylose content in the longer grain varieties. Toro and Century Patna are the exceptions in this regard, and they also differ in cooking quality from other long-grain rices. The poor cooking and processing characteristics of Century Patna 231 have been described in detail by Halick and Keneaster (*4*). Cooked samples of Toro are much more sticky and moist than other long-grain varieties, such as Rexoro and Bluebonnet. These findings (with the exceptions noted) indicate that amylose content may be responsible for the general processing characteristics of short-, medium-, and long-grain rices. It is, of course, premature at this point to lay emphasis on this hypothesis. Structural differences, genetic factors, and composition with regard to protein cannot be excluded until more data are obtained.

Comparison of Several Varieties Planted at Different Dates. Table II gives the percentage amylose for a number of varieties and experimental crosses obtained from the Rice Experiment Station, Crowley, La. These samples were grown under comparable field conditions so that there would be no variation resulting from soil type. There is no general trend for the amylose content to increase or decrease consistently with the date of planting (Table II). The first three varieties do show definite decreases which may reflect a response to date of seeding in those cases. Among the varieties, there is a consistent difference in amylose content along the general trend observed in Table I with the exception of Toro and Century Patna. The effect on amylose content of crossing grain types is interesting. Crossing Caloro, a pearl type, with Rexoro, a long-grain type, yielded a medium-grain rice of low amylose content. Likewise, in crossing Rexoro–Purple Leaf with Magnolia, a medium-grain rice of low amylose content was again obtained. On the other hand, in the crossing of Rexoro–Purple Leaf with "G" (long-grain rice), a long-grain rice of high amylose content resulted.

Table I. Varietal Differences in Amylose Content of Rice

Louisiana Samples, Variety	*Grain Type*	*Amylose, %*
Blue Rose Sel. 303	Medium	14.2
Toro 219	Long	15.5
Blue Rose 302	Medium	16.5
Fortuna 206	Long	17.3
Bluebonnet 204	Long	18.8
Rexark	Long	19.8
Bluebonnet-50-202	Long	22.2
Rexoro 401	Long	23.6
Sunbonnet 203	Long	23.6
Bluebonnet Sel. 208	Long	25.6

Texas Samples		*Amylose, %*		*Iodine Blue,*
Variety	*Grain Type*	*L.S.U.*	*S.U.R.D.D.*	*Value*
Century Patna 231	Long	12.9	13.6	73.0
Caloro	Pearl	14.3	13.8	42.0
Blue Rose	Medium	15.2	14.7	33.0
Bluebonnet 50	Long	21.5	20.7	24.0
Sunbonnet	Long	22.7	23.2	24.5
Texas Patna 49	Long	22.7	23.4	19.5
Rexoro	Long	25.0	22.8	20.0
Improved Bluebonnet	Long	23.2	22.5	17.5
Texas Patna	Long	23.4	23.1	17.0

Table II. Variation in Amylose Content with Date of Seeding

		Date of Seeding (1955)			
Variety	*Grain Type*	*3/8*	*4/26*	*5/14*	*6/20*
Century Patna	Long	14.2	13.8	13.5	12.1
Magnolia	Medium-long	14.0	13.4	12.8	11.9
Zenith	Medium	14.9	13.6	...	13.0
Caloro × Rexoro	Medium	14.1	15.1	13.0	12.9
Blue Rose Sel.	Medium	13.5	16.5	13.2	14.5
Nato[a]	Medium	13.5	14.0	13.2	13.5
Toro	Long	14.0	...	14.1	13.0
Rexoro with Delitus	Medium-long	20.5	21.6	21.7	23.2
Rexoro	Long	21.6	20.8	22.0	22.1
Bluebonnet-50	Long	21.1	20.2	22.2	26.1
Sel. 45 × 554[b]	Long	22.1	23.4	24.2	23.5
15/16 Rexoro	Long	22.7	23.0	23.0	22.9
Sunbonnet	Long	24.4	...	25.1	23.5

[a] Rexoro–Purple Leaf with Magnolia.
[b] Rexoro—Purple Leaf with "G."

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Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Methods of Analysis," 7th ed., p. 348, 1950.
(2) Bates, F. L., French, D., Rundle, R. E., *J. Am. Chem. Soc.* **65,** 142–8 (1943).
(3) Brown, F., Halsall, T. G., Hirst, E. L., Jones, J. K. N., *J. Chem. Soc.* **1948,** 27–32.
(4) Halick, J. V., Keneaster, K. K., *Cereal Chem.* **33,** 315–19 (1956).
(5) Hirst, E. L., Young, G. T., *J. Chem. Soc.* **1939,** 1471–82.
(6) Jodon, N. E., de la Houssaye, D. A., "Rice Varieties for Louisiana," Louisiana Bulletin No. **436,** 1949.
(7) McCready, R. M., Hassid, W. Z., *J. Am. Chem. Soc.* **65,** 1154–7 (1943).
(8) Schoch, T. J., *Ibid.*, **64,** 2957–61 (1942).
(9) Rao, B. S., Murthy, A. R. V., Subrahmanya, R. S., *Proc. Indian Acad. Sci.* **36B,** 70–80 (1952).

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